Citation:

Mennella JA, Pepino MY. Biphasic effects of moderate drinking on prolactin during lactation. *Alcoholism: Clinical and Experimental Research.* 2008; 32: 1,899-1,908. Epub: 2008 Aug 18.

PubMed ID: 18715274

Study Design:

Randomized control trial. 2x2 within-subject quasi-randomized trial

Class:

A - Click here for explanation of classification scheme.

Research Design and Implementation Rating:

POSITIVE: See Research Design and Implementation Criteria Checklist below.

Research Purpose:

To explore the effects of alcohol on suckling-induced prolactin levels and milk yield, and to determine the effects of alcohol on basal prolactin and prolactin response during lactation.

Inclusion Criteria:

- Female
- Lactating
- Exclusive breastfeeding of two- to five-month-old infants
- No resumption of menstruation
- Not pregnant throughout the study
- Blood glucose level less than 99mg per dL at the beginning of the study
- The University of Pennsylvania Office of Regulatory Affairs reviewed and approved the study. Participants gave written informed consent.

Exclusion Criteria:

- Non-lactating female
- Non-exclusive breastfeeding of two- to five-month-old infants
- Resumption of menstruation
- Pregnant
- Obese
- Smoker
- Anemic
- Alcohol-dependent
- Lifetime alcohol abstainer
- Previous gastric bypass surgery
- On any medication including oral contraceptives
- Participation in only one of the four test sessions.

Description of Study Protocol:

Recruitment

Recruitment procedures were not described.

Design

- The double-blind, two-by-two within-person, quasi-randomized trial consisted of four conditions:
  - Alcohol vs. no alcohol
  - Breast pumping once vs. twice
- Sessions were one week apart. The order of testing was counterbalanced so that the pumping condition alternated every two sessions, and the beverage alternated every session so that two weeks passed between alcohol conditions.

Blinding Used

Study nurses were blinded to the experimental conditions and participants were blinded to the alcohol conditions.

Intervention

- Participants completed the procedures at the same time for each session because prolactin concentration has a circadian rhythm. Women were asked not to drink alcohol for the three days prior to each session, and completed a pregnancy test prior to each session. During each session, participants were not allowed to sleep, talk about babies or food or watch television (because of possible exposure to babies or food) because of the possible effects on their hormones.
- After an overnight fast (verified by a fingerprick blood glucose assay), women completed baseline pumping to minimize discomfort. A nurse then inserted an intravenous line and the women acclimated in a private room for 45 minutes (needle pricks increase prolactin for 30 minutes).
- Blood samples were drawn at regular intervals before and after beverage consumption (-40, -25, -10, 25, 37, 39, 41, 43, 45, 47, 49, 51, 65, 80, 95, 110, 125, 140, 142, 144, 146, 148, 150, 152, 154, 156, 170, 185, 200 and 215 minutes). The beverage was divided into two equal volumes, each of which was to be consumed during consecutive five-minute intervals. The alcoholic beverage had a 0.4g per kg dose of ethanol in orange juice (15% v/v). The control beverage was an equal volume of orange juice. For both conditions, 3ml of alcohol was pipetted onto the cup surface to serve as a smell and taste mask.
- During the pump-once condition, women pumped 140 to 156 minutes after drinking. During the pump-twice condition, women pumped at 30 to 51 minutes and again at 140 to 156 minutes. The breast being pumped was alternated every two minutes for 16 minutes. Participants rated each breast's degree of fullness before and during each pumping session. Blood alcohol concentration (BAC) was also measured at fixed intervals during each session.
- After data collection finished at 215 minutes, subjects were provided lunch and were not allowed to leave the testing center until they had a BAC of zero.

Statistical Analysis

- BAC and prolactin (PRL) data preparation:
  - Peak BAC, time-to-peak BAC and alcohol disappearance rates (β_{60}; g per L per hour) were calculated using linear regression lines calculated within the apparent linear portion of the descending limb of each subject's BAC time curve. The first value was taken 30 minutes after peak BAC and all subsequent readings 0.20g per L or higher.
  - From each of the four sessions, peak PRL (mcg per L) and area under the PRL time curve (AUC; mcg per minute per L) was calculated for two time periods, 35 to 140 minutes and 140 to 215 minutes. AUC was determined using the trapezoidal method. PRL at 25 minutes was used as the baseline for delta scores for AUC at 35 to 140 minutes. PRL at 140 minutes was used as the baseline for delta scores for AUC at 140 to 215 minutes. Linear regression lines were calculated within the apparent linear portion of the ascending limb of the PRL rise for each time period for each beverage and pumping condition; correlation coefficients ranged from -0.75 to -1.00. One of six slopes could not be determined for one woman and three of six could not be determined for another.
- Hypothesis testing:
  - $P<0.05$ was used for all analyses
  - Slopes of the PRL rise were compared to determine whether alcohol consumption altered basal and suckling-induced PRL responses during the ascending and descending BAC
  - The effects of PRL response between feeds was determined by examining the effects of one pumping session on a later one. Alcohol effect modulation was also explored.
  - Separate repeated measures ANOVAs were run, using beverage and pumping conditions as well as time as within-subjects factors. Post-hoc Fisher's Least Significant Difference analyses were conducted for significant findings.
  - Secondary analyses explored effects of alcohol on milk yield, milk fat content and maternal perceptions

of breast fullness. Repeated measures ANOVAs were used from the first pump session.
- Correlations were used to identify associations between PRL, alcohol pharmacokinetics, and lactational performance.

Data Collection Summary:

Timing of Measurements

Blood samples were drawn before and after beverage consumption (-40, -25, -10, 25, 37, 39, 41, 43, 45, 47, 49, 51, 65, 80, 95, 110, 125, 140, 142, 144, 146, 148, 150, 152, 154, 156, 170, 185, 200 and 215 minutes). During the pump-once condition, women pumped 140 to 156 minutes after drinking. During the pump-twice condition, women pumped at 30 to 51 minutes and again at 140 to 156 minutes.

Dependent Variables

- Prolactin (PRL) concentration (mcg per L):
  - 2ml blood samples were drawn into containers with EDTA, kept on ice for up to an hour and centrifuged. Plasma aliquots were stored at -70°C. Plasma PRL was measured in duplicate with a direct two-site immunoradiometric assay.
  - PRL was measured in terms of baseline concentrations as well as suckling-induced response
- Milk yield (ml): Amount of milk pumped during each session
- Milk fat content (g per L): The creamatocrit method was used, where milk was vortexed, drawn into three microhematocrit tubes and centrifuged for 10 minutes. The height of the cream layer was measured and converted to fat by the formula, fat g per L = (mean creamatocrit percentage minus 0.59)/0.146
- Perceptions of breast fullness: Self-rated at the beginning and during pumping on a four-point scale (higher numbers mean more fullness).

Independent Variables

- Alcohol consumption (0.4g per kg dose vs. none)
  - BAC (g per L) measured by subjects breating into an Alco-Sensor IV at fixed intervals
  - BAC effects were assessed separately during ascending and descending BAC
- Pumping condition (pump once vs. twice).

Description of Actual Data Sample:

- *Initial N:* 15
- *Attrition (final N):* 13; two women completed only one session and were excluded from the analyses
- *Age:* 33.1±1.4 years
- *Ethnicity:* Six Caucasian, three African American, four other or mixed ethnicity
- *Anthropometrics:* Mean BMI of 24.7±1.1kg/m^2
- *Location:* University of Pennsylvania Clinical and Translational Research Center.

Summary of Results:

- BAC peaked around 42 minutes post-beverage at a concentration of 0.6±0.03g per L, and with a disappearing rate of 0.14±0.01g per L per hour
- Pumping significantly increased PRL. In the non-alcohol condition, PRL increased from 54.5±6.7mcg per L at baseline to 68.0±9.3mcg per L after 10 minutes of pumping and peaked at around 30 minutes at 106.7±15.9mcg per L. PRL levels were elevated for 75 minutes after pumping.
- Alcohol increased basal PRL compared to the no-alcohol condition ($P<0.0001$)
- During rising BAC, alcohol significantly enhanced PRL during breast pumping. The PRL slope was also steeper vs. the no alcohol condition ($P=0.07$)
- During falling BAC, alcohol significantly inhibited PRL during breast pumping, regardless of whether pumping was occurring for the first or second time. Peak PRL was significantly lower ($P<0.05$) and the PRL area under the curve at 140 to 215 minutes was significantly smaller ($P=0.05$). PRL slope was not significantly associated with beverage condition ($P=0.16$), pumping condition ($P=0.12$) or the interaction between the two ($P=0.95$).

- PRL dynamics were affected by both alcohol and pumping conditions
  - PRL levels were higher when women pumped for the second time vs. the first time. However, there were no differences in AUC at 140 to 215 minutes and slope of PRL rise between the pump conditions in the non-alcohol conditions. There were also no differences in the PRL time response.
  - Alcohol significantly delayed PRL response to pumping from eight minutes after pumping started to 12 minutes in the pump-twice condition or 14 minutes in the pump-once condition.

	Control			Alcohol		
	Pump Once	**Pump Twice**	**Once and Twice Combined**	**Pump Once**	**Pump Twice**	**Once and Twice Combined**
PRL AUC (mcg per minute per L)						
35- to 140-minute interval		2,653.5±835.5			4,153.2±1,224.3*	
140- to 215-minute interval	2,937.4±639.8	3,176.7±895.8	3,057.0±993.9	2,056.2±715.6	2,237.7±579.1	2,146.9±875.4**
Slope of PRL rise						
35- to 65-minute interval		2.6±0.7			3.8±1.0***	
140- to 170-minute interval	2.5±0.5	2.9±0.7	2.7±0.7	1.7±0.6	2.2±0.5	2.0±0.8
Peak PRL (mcg per L)						
35- to 140-minute interval		109.6±15.5			136.3±24.9	
140- to 215-minute interval****	98.5±15.7	132.1±22.7	115.3±25.5	85.4±17.2	107.8±16.8	96.5±23.2**

*P<0.05 for for same pumping condition, control beverage; **P≤0.05 for comparison with control beverage (both pumping conditions combined); ***P=0.07 for comparison with same pumping condition, control beverage; ****P<0.05 for comparison between pump-once (beverages combined) and pump-twice (beverages combined) (data not shown).

Other Findings

- Effects of time since pumping were assessed with the first pump session on each of the four days (35 to 51 minutes post-beverage to 120 minutes post-baseline pumping for pump-twice condition or 140 to 156 minutes post-beverage to 225 minutes post-baseline pumping for pump-once condition)
- Women reported feeling more breast fullness (1.4 vs. 1.0; P<0.001), expressed more milk (90.0 vs. 62.7ml; P=0.005), and had lower milk fat content (48.0 vs. 60.3g per L; P=0.06) in the pump-once condition vs. the pump-twice condition (i.e., longer interval since baseline pumping).

Author Conclusion:

- Breast pumping increased plasma prolactin concentrations in lactating women with infants age two to five months, with PRL returning to baseline in 75 minutes
- Alcohol modified PRL effects in a transient and biphasic manner

- Alcohol increased basal PRL within the hour after drinking, and the effects were stronger for lactating women than what has been observed previously with non-lactating women.
- During ascending BAC, alcohol enhanced the PRL response to pumping, but during descending BAC, it inhibited the PRL response
- Alcohol also altered PRL dynamics between pumpings. If a woman pumped within an hour of consuming alcohol, PRL response was delayed by a few minutes in a subsequent pumping session 1.5 hours later.

- Since the slope of PRL rise was steeper during pumping shortly after alcohol consumption, alcohol may be acting synergistically with PRL regulatory mechanisms. It is also unclear whether the effects are due to changes in PRL production or to changes in its metabolic clearance.

Reviewer Comments:

- *The authors did not describe any study limitations or potential biases*
- *As noted under study methods, randomization was done within fixed parameters for the two conditions so the condition order is only quasi-random. The study is currently class A, but if it were considered to be non-random assignment, it would be a class C study.*
- *Recruitment procedures were not described. Since the study was very intensive, it is likely that only highly motivated women participated. The participants are not likely to be representative of the population, but it is unclear how much of a concern this is since the study was of biological mechanisms rather than social or behavioral phenomena. For this reason, the selection of participants was determined to be free of bias on the research design and implementation checklist.*

Research Design and Implementation Criteria Checklist: Primary Research

Relevance Questions		
1.	Would implementing the studied intervention or procedure (if found successful) result in improved outcomes for the patients/clients/population group? (Not Applicable for some epidemiological studies)	N/A
2.	Did the authors study an outcome (dependent variable) or topic that the patients/clients/population group would care about?	Yes
3.	Is the focus of the intervention or procedure (independent variable) or topic of study a common issue of concern to nutrition or dietetics practice?	Yes
4.	Is the intervention or procedure feasible? (NA for some epidemiological studies)	Yes

Validity Questions		
1.	**Was the research question clearly stated?**	Yes
1.1.	Was (were) the specific intervention(s) or procedure(s) [independent variable(s)] identified?	Yes
1.2.	Was (were) the outcome(s) [dependent variable(s)] clearly indicated?	Yes
1.3.	Were the target population and setting specified?	Yes
2.	**Was the selection of study subjects/patients free from bias?**	Yes
2.1.	Were inclusion/exclusion criteria specified (e.g., risk, point in disease progression, diagnostic or prognosis criteria), and with sufficient detail and without omitting criteria critical to the study?	Yes
2.2.	Were criteria applied equally to all study groups?	Yes

2.3.	Were health, demographics, and other characteristics of subjects described?	Yes
2.4.	Were the subjects/patients a representative sample of the relevant population?	Yes
3.	**Were study groups comparable?**	Yes
3.1.	Was the method of assigning subjects/patients to groups described and unbiased? (Method of randomization identified if RCT)	Yes
3.2.	Were distribution of disease status, prognostic factors, and other factors (e.g., demographics) similar across study groups at baseline?	N/A
3.3.	Were concurrent controls used? (Concurrent preferred over historical controls.)	Yes
3.4.	If cohort study or cross-sectional study, were groups comparable on important confounding factors and/or were preexisting differences accounted for by using appropriate adjustments in statistical analysis?	N/A
3.5.	If case control or cross-sectional study, were potential confounding factors comparable for cases and controls? (If case series or trial with subjects serving as own control, this criterion is not applicable. Criterion may not be applicable in some cross-sectional studies.)	N/A
3.6.	If diagnostic test, was there an independent blind comparison with an appropriate reference standard (e.g., "gold standard")?	N/A
4.	**Was method of handling withdrawals described?**	Yes
4.1.	Were follow-up methods described and the same for all groups?	Yes
4.2.	Was the number, characteristics of withdrawals (i.e., dropouts, lost to follow up, attrition rate) and/or response rate (cross-sectional studies) described for each group? (Follow up goal for a strong study is 80%.)	No
4.3.	Were all enrolled subjects/patients (in the original sample) accounted for?	Yes
4.4.	Were reasons for withdrawals similar across groups?	N/A
4.5.	If diagnostic test, was decision to perform reference test not dependent on results of test under study?	N/A
5.	**Was blinding used to prevent introduction of bias?**	Yes
5.1.	In intervention study, were subjects, clinicians/practitioners, and investigators blinded to treatment group, as appropriate?	Yes
5.2.	Were data collectors blinded for outcomes assessment? (If outcome is measured using an objective test, such as a lab value, this criterion is assumed to be met.)	Yes
5.3.	In cohort study or cross-sectional study, were measurements of outcomes and risk factors blinded?	N/A
5.4.	In case control study, was case definition explicit and case ascertainment not influenced by exposure status?	N/A
5.5.	In diagnostic study, were test results blinded to patient history and other test results?	N/A
6.	**Were intervention/therapeutic regimens/exposure factor or procedure and any comparison(s) described in detail? Were interveningfactors described?**	Yes
6.1.	In RCT or other intervention trial, were protocols described for all regimens studied?	Yes

6.2.	In observational study, were interventions, study settings, and clinicians/provider described?	N/A
6.3.	Was the intensity and duration of the intervention or exposure factor sufficient to produce a meaningful effect?	Yes
6.4.	Was the amount of exposure and, if relevant, subject/patient compliance measured?	Yes
6.5.	Were co-interventions (e.g., ancillary treatments, other therapies) described?	N/A
6.6.	Were extra or unplanned treatments described?	N/A
6.7.	Was the information for 6.4, 6.5, and 6.6 assessed the same way for all groups?	Yes
6.8.	In diagnostic study, were details of test administration and replication sufficient?	N/A
7.	**Were outcomes clearly defined and the measurements valid and reliable?**	Yes
7.1.	Were primary and secondary endpoints described and relevant to the question?	Yes
7.2.	Were nutrition measures appropriate to question and outcomes of concern?	N/A
7.3.	Was the period of follow-up long enough for important outcome(s) to occur?	Yes
7.4.	Were the observations and measurements based on standard, valid, and reliable data collection instruments/tests/procedures?	Yes
7.5.	Was the measurement of effect at an appropriate level of precision?	Yes
7.6.	Were other factors accounted for (measured) that could affect outcomes?	Yes
7.7.	Were the measurements conducted consistently across groups?	Yes
8.	**Was the statistical analysis appropriate for the study design and type of outcome indicators?**	Yes
8.1.	Were statistical analyses adequately described and the results reported appropriately?	Yes
8.2.	Were correct statistical tests used and assumptions of test not violated?	Yes
8.3.	Were statistics reported with levels of significance and/or confidence intervals?	Yes
8.4.	Was "intent to treat" analysis of outcomes done (and as appropriate, was there an analysis of outcomes for those maximally exposed or a dose-response analysis)?	N/A
8.5.	Were adequate adjustments made for effects of confounding factors that might have affected the outcomes (e.g., multivariate analyses)?	???
8.6.	Was clinical significance as well as statistical significance reported?	Yes
8.7.	If negative findings, was a power calculation reported to address type 2 error?	N/A
9.	**Are conclusions supported by results with biases and limitations taken into consideration?**	Yes
9.1.	Is there a discussion of findings?	Yes
9.2.	Are biases and study limitations identified and discussed?	Yes
10.	**Is bias due to study's funding or sponsorship unlikely?**	Yes
10.1.	Were sources of funding and investigators' affiliations described?	Yes

10.2.	Was the study free from apparent conflict of interest?	Yes